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Morphological aspects and pathological impacts of cystic echinococcosis in liver of slaughtered sheep and host–parasite relationship

[*Aspectos morfológicos e impactos patológicos da equinococose cística no fígado de ovelhas abatidas e relação hospedeiro-parasita*]

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ABSTRACT

Cystic echinococcosis (CE) is a parasite–borne disease caused by the larval stage of *Echinococcus granulosus* belonging to family Taeniidae, is an endemic disease, distributed worldwide as a neglected parasite with socioeconomic repercussions. It causes severe alterations in liver of infected host, and it could be fatal if untreated. The main objective of the present study is to measure and evaluate the condition of hydatidosis in slaughtered sheep in Al Baha region, Saudi Arabia. A total of 601 slaughtered sheep in Al Baha abattoirs were examined for hydatidosis during the year 2020. Various organs were examined for the presence of parasite cysts, and the cyst contents were examined by light microscopy. Also, liver sections infected with cysts were examined for pathological alterations and host–parasite interactions. Forty-two animals were infected (6.09%) with the highest prevalence during summer. During gross examination, it was observed that the sheep liver is the most affected organ by the disease. The liver contained multiple cysts with varying sizes, filled with clear to slightly turbid fluid containing protoscoleces. Detailed sections of the infected liver revealed significant fibrosis around the central vein, portal vein congestion, infiltration of inflammatory cells, and necrosis. Sections through hydatid cysts showed the presence of three surrounding layers with a laminar membrane bordering the cyst lined by a germinal epithelium surrounding several brood capsules with many protoscolesces inside supported by a set of rostellar hooklets. Considerable efforts should be spent to control the transmission of parasitic cysts and inhibit the incorrect disposal of infected meat from slaughterhouses. Also, more focus should be done on epidemiological and control programs of these parasites.

Keywords: Echinococcosis, brood capsule, laminar membrane, morphology, histopathology

RESUMO

A equinococose *cística (EC) é uma doença transmitida por parasitas causada pelo estágio larval do* Echinococcus *granulosus, pertencente à família Taeniidae, é uma doença endêmica, distribuída mundialmente como um parasita negligenciado com repercussões socioeconômicas. Ela causa alterações graves no fígado do hospedeiro infectado e pode ser fatal se não for tratada. O principal objetivo do presente estudo é medir e avaliar a condição da hidatidose em ovelhas abatidas na região de Al Baha, na Arábia Saudita. Um total de 601 ovelhas abatidas em matadouros de Al Baha foram examinadas quanto à hidatidose durante o ano de 2020. Vários órgãos foram examinados quanto à presença de cistos de parasitas, e o conteúdo dos cistos foi examinado por microscopia de luz. Além disso, seções de fígado infectadas com cistos foram examinadas quanto a alterações patológicas e interações hospedeiro-parasita. Quarenta e dois animais foram infectados (6,09%), com a maior prevalência durante o verão.*

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Durante o exame macroscópico, observou-se que o fígado das ovelhas é o órgão mais afetado pela doença. O fígado continha vários cistos de tamanhos variados, preenchidos com fluido claro a levemente turvo contendo protocistos. Seções detalhadas do fígado infectado revelaram fibrose significativa ao redor da veia central, congestão da veia porta, infiltração de células inflamatórias e necrose. As seções através dos cistos hidáticos mostraram a presença de três camadas circundantes, com uma membrana laminar que margeia o cisto, revestida por um epitélio germinativo que envolve várias cápsulas de cria com muitos protoscolescentes em seu interior, sustentados por um conjunto de ganchos rostelares. Esforços consideráveis devem ser despendidos para controlar a transmissão de cistos parasitários e inibir o descarte incorreto de carne infectada em abatedouros. Além disso, deve-se dar mais atenção aos programas epidemiológicos e de controle desses parasitas.

Palavras-chave: equinococose, cápsula de cria, membrana laminar, morfologia, histopatologia

INTRODUCTION

Echinococcosis (hydatidosis) is an important zoonotic disease in many parts of the world, including Saudi Arabia, and it poses a great threat to human health and livestock as an endemic disease (Almalki *et al.,* 2017; Toulah *et al.,* 2017). Information and epidemiology regarding the hydatidosis in livestock in the Middle Eastern region including Saudi Arabia is particularly limited (Almalki *et al.,* 2017; Mofleh *et al.,* 2023). The larvae and adult stages of cestodes belonging to the genus *Echinococcus* and the family Taeniidae are the main culprits behind the clinical symptoms of the disease (Abu-Eshy, 1998). The severe clinical forms of this disease are caused by the larval and adult stages of cestodes of the genus *Echinococcus* and the family Taeniidae (Eckert and Deplazes, 2004). There are 9 valid species of the genus *Echinococcus* that have been identified: *E. granulosus* (*sensu strict*) (sheep strain, Tasmanian sheep strain and buffalo strain); *E. ortleppi*; *E. canadensis*; *E. equinus*; *E. felidis*; *E. vogeli*; *E. multilocularis*; *E. oligarthrus*; and *E. shiquicus* (Addy *et al.,* 2017; Nakao *et al.,* 2013; Saarma *et al.,* 2009). *E. granulosus* (*s.s.*) and *E. multilocularis* represent the most important species responsible for human CE (Adewunmi & Basilingappa, 2004). The normal life cycle of *Echinococcus* species includes two mammalian hosts; adult worms inhabit the small intestine of canids as definitive hosts, while larval stages or the hydatid cyst occur in tissues of herbivorous livestock and wild ungulates as intermediate hosts and, occasionally, in humans who can be infected after ingestion of parasite eggs (Mofleh *et al.,* 2023; Romig *et al.,* 2015). Due to increased interaction between dogs and domestic animals in rural regions, livestock serves as the primary source for transmitting the disease to humans (Almalki *et al.,* 2017). The disease has a noteworthy influence on the economy and public health of countries where the expansion of pastures constitutes the primary basis for animal production, especially in regions where livestock rearing is a significant component of agriculture. Thus, it is essential to identify the prevalence of infection in intermediate hosts in order to determine to what extent the parasite spreads (Cadavid Restrepo *et al.,* 2016). The infection with this parasite in intermediate hosts is asymptomatic and it is difficult to diagnose the infection in sheep, camels, cattle, goats by external investigation (Hayajneh *et al.,* 2014). Cyst detection can be performed at postmortem inspection and during meat investigation in the slaughterhouse. The domestic sheep in Saudi Arabia is the major source of milk, meat, and straight wool (Ali & Al-Noaim, 1992), which didn't receive any attention in the northwest part of Saudi Arabia. In the present study, the condition of hydatidosis in slaughtered sheep in the Al Baha region, Saudi Arabia was evaluated; based on prevalence, light microscopy and histopathology.

MATERIALS AND METHODS

Examination of the slaughtered sheep: this work was conducted by post–mortem examination of sheep slaughtered in Al Baha region slaughterhouses in the southwest of Saudi Arabia (20.0217° N, 41.4713° E). A total of 601 animals were investigated for the presence of hydatid cysts during the year of 2020. Data including age, sex and site of infection were recorded for each animal. At the slaughterhouse, slaughtered sheep were examined visually for palpation of hydatid cysts in various organs notably the liver, lungs, heart and kidneys according to (Multicriteria…, 2014). Infected organs were transported in ice boxes to the Laboratory of Parasitology, Biology Department, College of Science, Al Baha University, where data such as cyst dimensions were collected.

Light microscopy: The fertility and viability of cysts were evaluated by investigating the protoscolices. Cyst fertility was determined by isolating and rupturing the cysts and collecting the transparent fluids using a sterile syringe. These fluids were then scrutinized under a microscope for the presence of protoscoleces. Protoscolices viability was determined according to Moazeni and Larki, 2010 by staining in 0.1% eosin solution; viable protoscolices do not take up the stain, whereas the non–viable ones do. Protoscolices and their hooks were photographed with a BX53 microscope (Olympus Corporation, Japan).

Histopathological Study: cyst fertility and viability of protoscolices were investigated; the cyst fertility was measured through isolation and rupture of cysts. The transparent fluids were aspirated by a sterile syringe and examined microscopically for the presence of protoscoleces. Protoscolices viability was determined according to Moazeni & Larki, 2010 by staining in 0.1% eosin solution, viable protoscolices do not take up the stain, whereas the non–viable ones do. Protoscolices and their hooks were photographed with a BX53 microscope (Olympus Corporation, Japan).

RESULTS

Prevalence of infection: It was shown that the infection was spread throughout the year, the overall prevalence of infection was 6.09% (42/601), the highest prevalence was recorded in summer (8.55%: 23/269), while the lowest prevalence was reported in winter (5.72%: 19/332) with no significant difference between seasons. The results also showed that infection differed according to the age of the examined sheep, the highest prevalence was recorded in sheep aged 6–12 months (3.28%: 20/601), followed by sheep aged 13–24 months (2.33%: 14/601) while the lowest prevalence was in sheep aged more than 2 years (1.33%, 8/601).

Gross pathology: The sheep's visceral organs were visually inspected for any visible changes caused by cysts, and it was found that liver tissues were the most affected organ with a prevalence of 71.43% (30/42) followed by lungs with a prevalence of 19.05% (8/42), with the lowest prevalence recorded as a concurrent infection of liver, lungs and other visceral organs with a rate of 9.52% (4/42). The infection was observed as single to multiple cysts of various sizes (Figure 1a–c). Hepatic cysts were either invisible and completely embedded deep in the liver parenchyma or partially embedded and protruding from the liver surface of infected lobes. The fertile cysts were observed to have a thick, soft, smooth, and creamy white laminated layer filled with a clear or slightly turbid fluid. Non–fertile cysts had a thin yellow laminated layer tightly attached to the lesion, where some cysts were slightly calcified and hard to be processed.

Light microscopy: After rupture of cyst, many protoscoleces were generated (Figure 1d–g), each protoscolex has a spherical body with approximately 90×75µm diameter with an invaginated scolex equipped by hooks, each hook was 20µm in diameter (Figure 1h,i). The fertility of cysts was investigated by the presence of free protoscolices in the fluid through wet mount drop examined by light microscopy. For the determination of viability of protoscolices, it was found that dead protoscolices stained red as they absorbed eosin, they appeared morphologically distorted with some degeneration observed. Alive protoscolices remained unstained with flame cell activity.

Histopathology: Histopathological studies of the control sheep liver (Figure 2a–c) showed normal hepatic architecture and normal morphology of hepatic lobule with normal central vein bounded

by a normal connective tissue. Parallel hepatic cords of hepatocytes radiated from the central vein towards the periphery of the hepatic lobule and are separated by sinusoidal spaces and normal connective tissue around the portal space "triad". The histological sections from liver of sheep infected with hydatide cysts showed an increase in collagen fiber deposition "fibrosis or cirrhosis around the central vein and in the portal space "triad" (Figure 2d–f). Examination of the hepatic tissue indicated the presence of portal and central vein congestion, characterized by an infiltration of lymphocyte cells and an increase in the deposition of collagen fibers forming a broad fibrous layer of connective tissue encircling the portal and central veins. Additionally, inflammation around the portal area and expansion of the portal vein were also observed. Hepatocytes of the liver parenchyma neighboring the cyst wall revealed degenerative changes, necrosis, infiltration of inflammatory cell mainly lymphocytes together with broad fibrosis around portal area in the hepatic tissue surrounding the hydatid cysts (Figure 2d–g). Sections through hydatid cysts showed that each cyst was bordered by a cyst wall which was composed of three layers (Figure 3a–c); Pericyst or ectocyst (outer adventitial layer) in the form of fibrous and granular tissues as well as blood vessels. Exocyst or laminated membrane, it was tough and elastic non–cellular hyaline layer of variable thickness (Figure 3d–f). This layer was similar to the white of a hardboiled egg. The third inner layer; the Endocyst or the germinative/germinal layer which was about 25μm thickness (Figure 3g–i), was a nucleated layer, nuclei embedded in a protoplasmic mass, actively producing both laminated membrane outward and the brood capsules inwards. The cyst lumen is lined by acellular folded fibrous laminated membranous structures with characteristic concentrically chitinous layers with a prominent striation, which were dense pinkish in H&E stained sections, bluish in Mallory trichrome stained sections, and light pinkish in PTAH stained sections. The laminar membrane bordering the cyst was lined by a germinal epithelium. The germinal layer consisted of eosinophilic layer which produces several daughter cysts called brood capsules (Figure 4a–e) within the hydatid sand inside which protoscoleces grown. Each brood capsule usually contained a cluster of protoscolesces (6 –10) with a set of rostellar hooklets (Figure 4f).

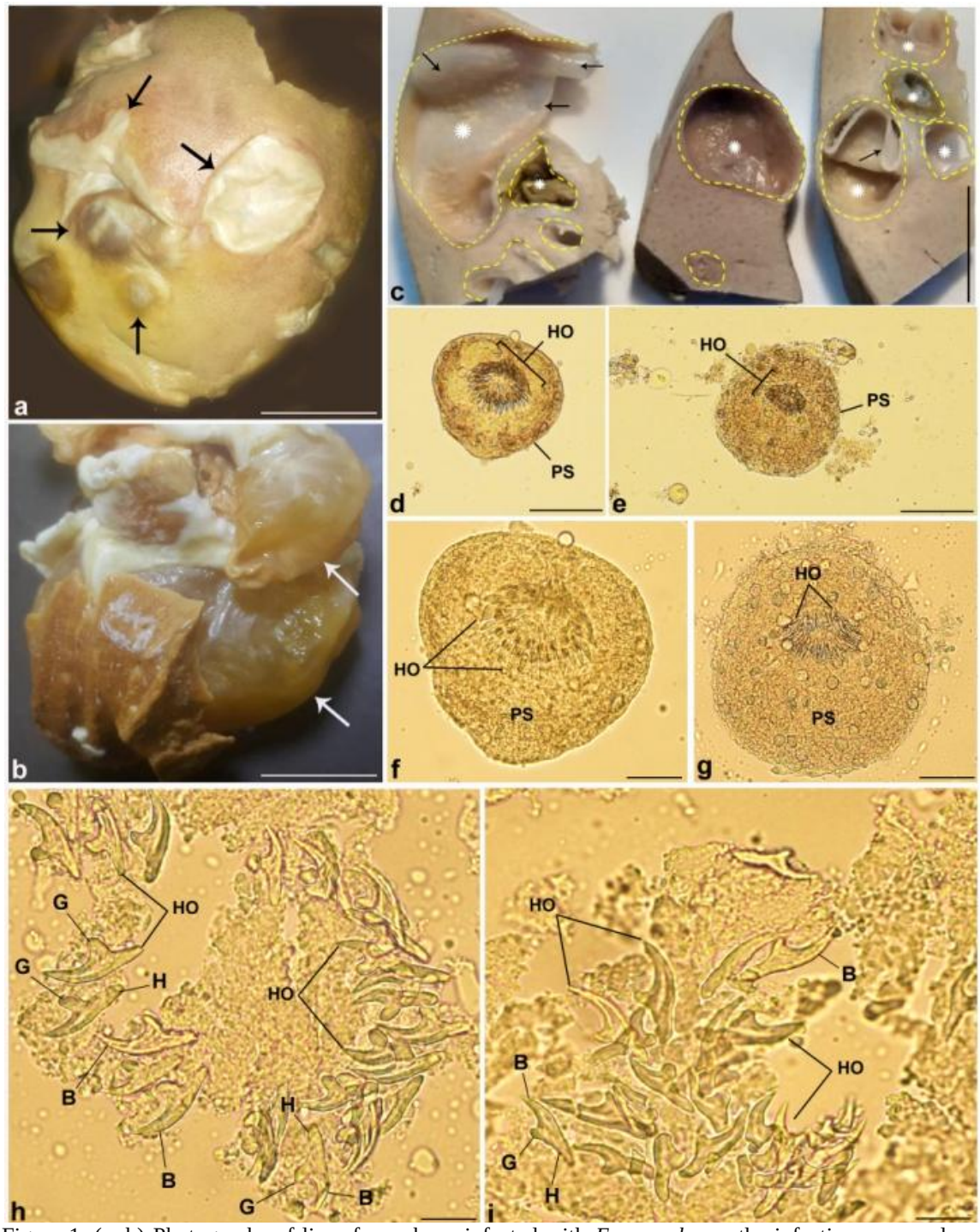

Figure 1. (a, b) Photographs of liver from sheep infected with *E. granulosus*, the infection appeared as multiple cysts of varying sizes (arrows), Bars 2cm; (**c)** Cross sections through liver of sheep infected with *E. granulosus* as round to oval variable sized cystic lesions (asterisks) bordered by a detached folded whitish gelatinous membrane (arrows), Bar 2cm; (**d–g)** Photomicrographs showing the morphological aspect of the cyst Protoscolices (PS) with double row hooks (HO), Bars, d, g 50µm, f, g 20µm; (**h–i)** Photomicrographs of rostella hooks (HO) from a protoscolex, showing the characteristic morphological features of the handle (H), guard (G) and blade (B) of each hook, Bars 5µm.

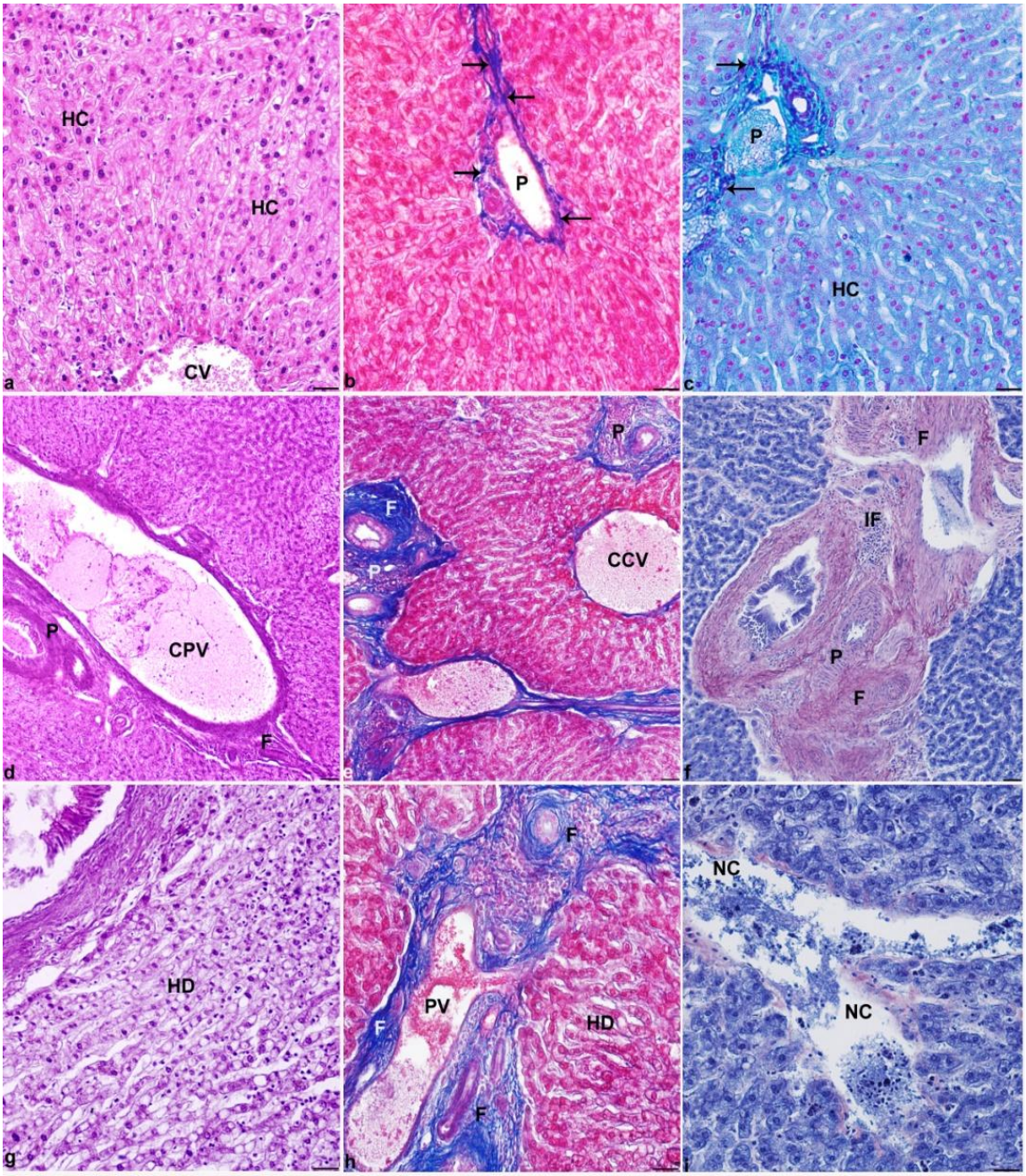

Figure 2. (a–c). Photomicrographs for sections through liver of control sheep showing the normal morphology of hepatic lobule with normal central vein (CV). Parallel hepatic cords of hepatocytes (HC) radiate from the central vein towards the periphery of the hepatic lobule with normal connective tissue (arrows) around the portal space "triad" (P), Bars 100µm; (**d–f)** The histological alterations in liver sections from infected sheep, they exhibited an increase in collagen fiber deposition "fibrosis (F)" around the central vein and in the portal space "triad" (P). Marked dilation and congestion of central (CCV) and portal (CPV) veins in addition to the release of many inflammatory cells (IF) around the portal area, Bars 200µm; (**g–i)** Sever hydronic degeneration (HD) and fibrosis around the portal vein (PV) as well as prominent necrosis (NC) in liver sections from infected sheep, Bar 100µm. a, d, g H&E stained, b, e, h MT stained, c, f, i PATH stained.

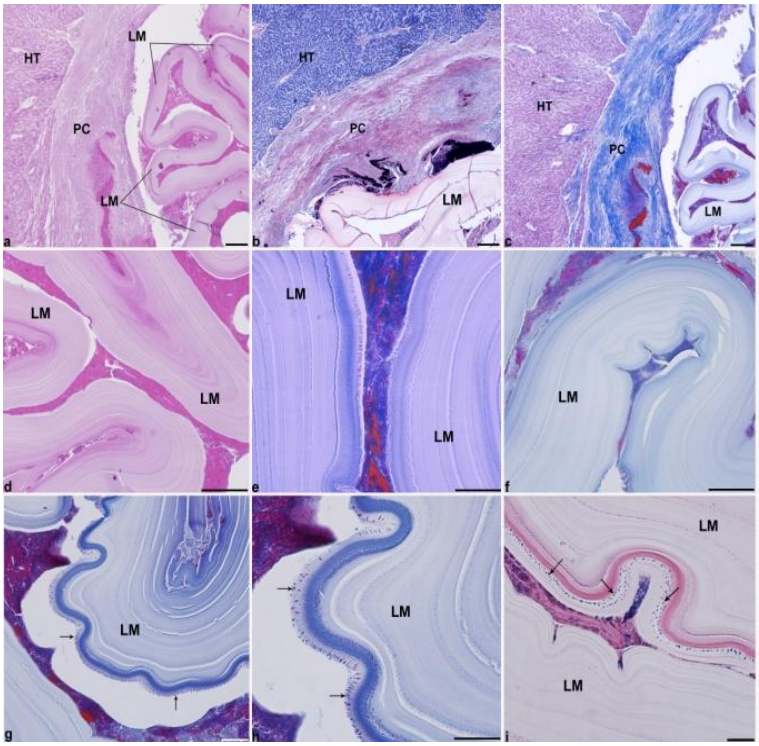

Figure 3. Photomicrographs of sections through hydatid cyst of *E. granulosus* showing the typical layering of the cyst wall, three layers are observed following the host tissue (HT), the outer adventitial layer (PC), the laminated membrane (LM) which is acellular hyaline layer and lined by the germinal layer (arrows) it was a nucleated layer, nuclei embedded in a protoplasmic mass, actively producing both laminated membrane outward and the brood capsules inwards. Bars, a–d, g, h, i 200µm, e, f 100µm.

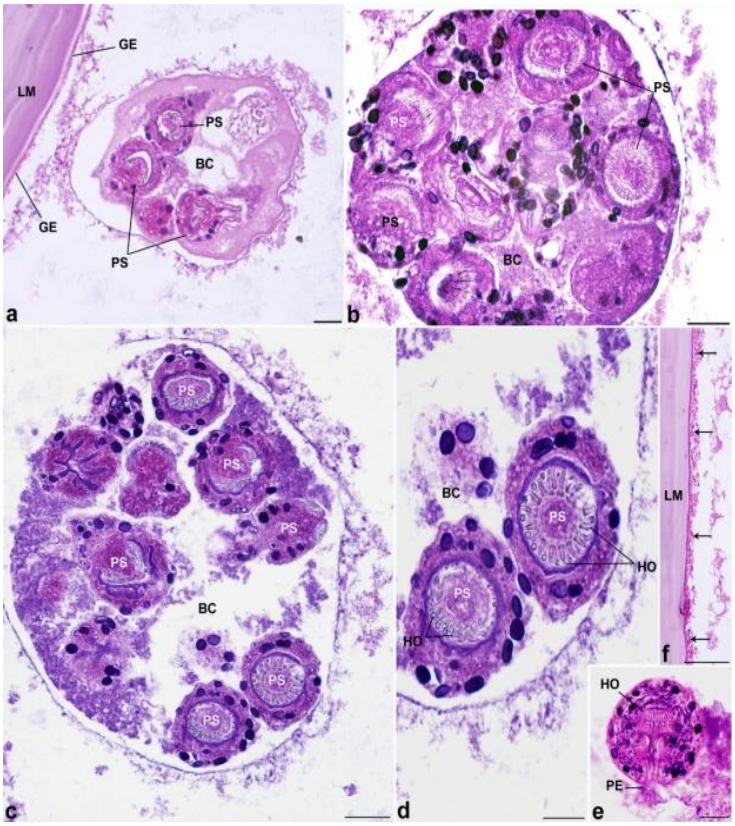

Figure 4. (a–e). Photomicrographs of sections through hydatid cysts isolated from infected sheep, the cyst wall consists of acellular laminated membrane (LM, high magnification in fig. f) lined by germinal epithelium (GE), from which several Brood capsules (BC) arises, inside these capsules, cluster of protoscoleces were projected with rows of rostellar hooks (HO), each scolex is attached through a pellicle (PE). Bars, a–e 25µm, f 10µm.

DISCUSSION

Cystic echinococcosis is a worldwide geographically distributed disease with a wide variety of hosts (Multicriteria…, 2014). It is important to monitor this disease by collecting current data in animals and humans to track its epidemiology. It was found that most of the infections recorded herein by this parasite were recorded in liver followed by lungs while the concurrent infection was the lowest prevalence, this is in accordance with previous studies mentioned that liver tissue is the first organ into which blood flows from the digestive tract (Ibrahim, 2010; Toulah *et al.*, 2017). Also, liver has been reported as one of the most favored sites for hydatid cyst development followed by lungs, other internal organs such as skeletal muscles, nervous system and bone marrow may also be infected (Almalki *et al.,* 2017; Cadavid Restrepo *et al.,* 2016). Previous numerical records concerning the prevalence of hydatidosis in sheep inhabiting different regions in Saudi Arabia were performed such as those of Ibrahim (2010) in Al Baha region with a prevalence of 12.61%; Toulah *et al.* (2017) in Jeddah, 69.6% and Hayajneh *et al.* (2014) in Al Taif, 13.5%. The Kingdom of Saudi Arabia is known to import livestock in a large–scale from other Middle Eastern and African countries where the disease is highly prevalent; this explained the high infection rate by this parasite (ElGhareeb, 2017; Fdaladdin *et al.*, 2013). The present study was carried out in sheep where livestock hosts such as sheep, goats and camels are the main reservoirs of cystic diseases which are of public health significance and economic importance in Saudi Arabia (Abdel-Baki *et al.*, 2016; Hussein *et al.,* 2012). The high prevalence rate recorded in sheep may be related to the feeding habit where the grazing sheep are the most exposed animal to get cestode eggs during feeding (Beigh *et al.*, 2017). In the current study, the prevalence of infection with hydatidosis revealed fluctuation between summer and winter seasons, with a maximum prevalence recorded in summer (8.55%) then decreased to a lower prevalence in winter (5.72%). The results agreed with Ernest *et al.* (2009) and Almalki *et al.* (2017), they claimed that seasonal variation in infection may be related to the differences in the environmental conditions that cause fluctuations in the spreading of the parasite, the availability of final hosts, and the nature of the pasture among seasons. Gross morphology showed that infected livers have single to multiple cysts of various sizes, they are partially or fully embedded in the liver parenchyma and distributed randomly through their lobes. Similar observation has been reported previously (Anwar and Tanveer, 1999). The recorded liver cysts were soft with clear to slightly turbid fluid; similar record had been reported by (Ibrahim, 2010). In terms of histological changes, liver sections from infected sheep showed numerous pathological alterations compared to the control group. These findings were consistent with previous research which indicated that infected visceral organs display a range of histological changes and host responses (Hamrat *et al.,* 2011; Miman *et al.,* 2009). In the present study, an outer adventitial layer was observed encapsulating the cyst; it consists of fibrous and granulated tissues surrounded by inflammatory cells. This observation coincides with the study of Singh *et al.* (2016) who concluded that following infection with CE, a fibrous capsule is formed around the parasite as a cellular response from the intermediate host; this layer enlarges as the cyst grows. The displacement of liver tissue by the parasite cyst as well as the pressure caused on liver as the cyst size increases leads to pathological tissue changes, previous studies such as those of Al Se´adawy and AlKaled, 2012; Torgerson and Deplazes, 2009 mentioned that the compact tissue and the inflammatory zone formed around the cyst may resists the development of larger cysts and favors cyst calcification. The current investigation showed that upon examining the histological sections of liver and parasite cysts, a tri-laminar membrane with germinal epithelium lining, comprising of an eosinophilic layer, was identified. Additionally, multiple protoscoleces were observed within brood capsules, these in accordance with Cao *et al*. (2021) and Yasen *et al*. (2021), they mentioned that the hydatid cyst wall from inside to outside is composed of germinal layer, pericyst and a laminated membrane. The space observed between the pericyte and the laminated membrane resembles those recorded in the study of (Kul and Yildiz, 2010) who conclude that through this space, tissue fluids and the nutrient medium can flow. Congestion, hemorrhage and cellular infiltrations were observed mostly in the inner side of the fibrous capsule in accordance with Singh *et al.*, 2016. Necrosis in infected tissue may be attributed to the loss of blood supply to the

infected tissues, Miman *et al*. (2009) claimed that necrosis was due to thrombosis of the vessels in the host tissue around the cyst and so all necrotic masses observed in the infected liver must include the parasite. Further, the extensive fibrosis observed in some areas was attributed to host immunological reactions (Hamrat *et al.*, 2011). Histological observations of hydatid cysts from infected sheep showed that the recorded cysts belongs to *E. granulosus* according to the key published by Thompson and McManus, 2002 depending on the shape and arrangement of laminar membrane, the nucleated germinal epithelium, and the pathological response of the host toward the parasite cyst.

CONCLUSION

Histopathological as well as gross examinations are important tools for postmortem diagnosis of CE, however, more focus on epidemiological monitoring, surveillance and control programs should be performed on economically important animals. Also, considerable efforts should be spent to control the transmission of parasite cysts and inhibit the incorrect disposal of infected meat from slaughterhouses.

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